

about 8 different C genes. None of the publications of record suggests which of these many separate regions on regions genes should be deleted or inactivated so as to result in the inability of the locus to rearrange or to produce a functional message encoding a immunoglobulin heavy-chain subunit. Furthermore, prior to the work of the Applicants, it was unclear if inactivation of part of the heavy chain locus would simply be compensated for by non-conventional rearrangements during B cell development.

The following paragraphs are a summary of some of the differences between the publications cited in the draft office action. These differences were discussed in the interview on December 6, 1995.

Publications Cited In The Draft Office Action

1. Koller (U.S. Patent No. 5,416,260) describes work used to obtain mice that do not produce MHC class I protein by virtue of inactivation of the $\beta 2$ microglobulin gene. Koller describes the use of homologous recombination and gene targeting to inactivate the $\beta 2$ microglobulin gene. Individual cells produce several different types of MHC class I protein; however, each different MHC class I protein is complexed with the $\beta 2$ microglobulin protein. By targeting the $\beta 2$ microglobulin gene, Koller sought to interfere with the expression of the various MHC class I proteins. $\beta 2$ microglobulin is a small (12 kd) protein encoded by a small gene with well-defined exon-intron boundaries. The $\beta 2$ microglobulin gene was extremely well characterized prior to Koller's attempts to inactivate the gene. In contrast, the mouse immunoglobulin genes are much more complex than the $\beta 2$ microglobulin gene. The immunoglobulin heavy chain locus is about 2500 kb in length. Moreover, the entire structure of the human heavy immunoglobulin locus is still not characterized or fully understood. Furthermore, unlike the J region which consists of several separate J encoding genes (or segments), the $\beta 2$ microglobulin gene is a single gene.

It is further noted that Koller inactivates the $\beta 2$ microglobulin gene by inserting a G418 resistance cassette

into the gene. The introduction of an insertional inactivation cassette into the J region of the mouse immunoglobulin genes would not have resulted in inactivation of the gene because of the multiple copies of each, J gene at the heavy chain locus.

Furthermore, it is noted that Koller does not describe the targeting of an immunoglobulin gene, much less the targeting of the J region.

2. Miller et al. (hereinafter "Miller") describes the organization of the mouse λ light-chain genes. Miller merely describes the organization of the naturally occurring mouse λ light chain J region. Miller contains no description of experiments in which the mouse immunoglobulin genes are structurally altered. Miller merely discovered that λ J4 (one of several J genes in the J region) is a pseudogene that is not expressed. The lambda J4 region is shown to contain a 2 base pair deletion. The fact that the λ J4 region has mutations is thought to be the reason that no λ light chain molecules containing the λ J4 region are expressed; however, Miller does not contain any experiments that test this proposition. Miller is only concerned with one of the several J regions in the λ light-chain mouse immunoglobulin. No teachings are made as to the alteration of the structure of other J regions or all of the J regions. Furthermore, Miller does not include any suggestion that any or all J regions in the light chain or heavy chain be intentionally deleted. Moreover, as the λ light-chain does rearrange in mice, the mutation in the J 4 region clearly does not block rearrangement of the λ light-chain immunoglobulin gene. Furthermore, Miller contains no teaching about mutations in the J region of the immunoglobulin heavy chain subunit gene.

3. Lorenz et al. (hereinafter "Lorenz") concerns the physical map of the human κ immunoglobulin light-chain. The map provided is obtained by pulsed-field gel electrophoresis and is extremely crude. The map does not provide sufficient detail to permit the isolation and cloning of the J regions of

the κ gene, much less the J regions of the heavy chain immunoglobulin gene. Furthermore, Lorenz contains no suggestion that the J region of an immunoglobulin heavy chain locus should be inactivated.

4. Bruggemann et al. (hereinafter "Bruggemann") describes an experiment in which a small portion of the human immunoglobulin heavy chain locus is introduced into transgenic mice. Bruggemann contains no description of how to inactivate endogenous immunoglobulin genes, much less any specific teaching that the J region of the immunoglobulin heavy chain gene should be targeted. Additionally, it should be noted that Bruggemann does not teach how to insert large portions of unrearranged immunoglobulin genes into transgenic mice.

5. Krimpenfort et al. (U.S. Patent No. 5,175,384, hereinafter "Krimpenfort") describes a method of producing transgenic animals that are impaired in T cell development. Krimpenfort introduces a modified rearranged T cell receptor gene into mice. The expressed modified rearranged T cell receptor gene results in the ablation of T cell development in the transgenic mice. Krimpenfort does not target the endogenous T cell receptor genes, instead, Krimpenfort expresses a modified rearranged T cell receptor gene from an untargeted site within the mouse genome. Thus, Krimpenfort does not teach that any endogenous locus must be targeted to achieve the desired objective.

The clear goal of Krimpenfort is to destroy T cell development. Krimpenfort contains speculation, but no actual experimentation, that an analogous technique could be used to impair B cell development, i.e. the expression of a modified rearranged immunoglobulin gene. Thus, Krimpenfort actually teaches away from the claimed invention. If Krimpenfort's speculative technique for destroying B cell development was to actually work, then there would be no need to inactivate immunoglobulin gene expression because immunoglobulin expressing B cells would never form in the first place. Moreover, no xenogenic (human) immunoglobulin DNA could be

expressed because of the non-functional B cells in the transgenic animals taught in Krimpenfort.

Furthermore, Krimpenfort actually seeks to express the transgene inserted, whereas Applicants manipulation of the endogenous J region is performed in order to inactivate expression of the endogenous immunoglobulin heavy-chain subunit by introducing a lesion (or lesions) into the J locus.

SUMMARY

In view of the above discussion of the cited publications, it apparent that none of these publications, taken alone or in combination with each other, teach or suggest that the J region of an immunoglobulin locus should be deleted so as to prevent the formation of a functional message encoding a heavy-chain subunit. Accordingly, all pending rejections should be withdrawn and the application be allowed to issue.

Respectfully submitted,

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Dated: 12/28/95

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Enclosure